Journal of Medical Virology 60:205–209 (2000)

Cidofovir, a New Approach for the Treatment of Cervix Intraepithelial Neoplasia Grade III (CIN III)

Robert Snoeck,[1]* Jean-Christophe Noel,[2] Christine Muller,[3] Erik De Clercq,[1] and Michel Bossens[3]

[1]Rega Institute for Medical Research, Leuven, Belgium
[2]Pathology Department, Erasme Hospital, Free University of Brussels, Brussels, Belgium
[3]Gynaecology Department, Centre Hospitalier Etterbeek-Ixelles, Brussels, Belgium

Cervix intraepithelial neoplasia grade III (CIN III) is an intraepithelial proliferative process with different levels of severity depending on both the extension of the proliferation in the epithelium and the presence of cellular atypia. Human papillomavirus (HPV) has been clearly associated with such lesions. The results of a preliminary study are described on the local application of cidofovir, an acyclic nucleoside phosphonate derivative with broad-spectrum anti-DNA virus activity for the treatment of CIN III. Cidofovir 1% in gel was applied three times, every other day, on the cervix of each of 15 women with biopsy proven CIN III. Within 1 month after the start of treatment, the cervix was removed surgically. Histology and human papillomavirus polymerase chain reaction (HPV-PCR) were carried out. In 7 of the 15 patients the histology showed a complete response, whereas 5 patients had a partial response characterized by the persistence of CIN II-III lesions, 1 patient had a dysplasia of lower grade (CIN I), and 2 patients did not show differences in the histology. Complete response was confirmed by PCR in 4 of the 7 patients, with complete response histologically. Cidofovir was not toxic to the normal epithelium. Cidofovir 1% gel was able to inhibit partially or completely cervical dysplasia lesions after only three applications (every other day). This effect was specific and tissue other than the dysplastic epithelium was not affected by the treatment. ***J. Med. Virol. 60:205–209, 2000.***

KEY WORDS: cervical intraepithelial neoplasia; human papillomavirus; cidofovir

INTRODUCTION

Cervix intraepithelial neoplasia (CIN) grade III is the most advanced histological stage of cervical dysplasia, graded according to the proliferative intraepithelial squamous lesions that display abnormal maturation, nuclear enlargement, and atypia. In CIN III, the full thickness of the epithelium is occupied by undifferentiated basal-type cells. Disruption of the basal membrane by the tumour cells leads to microinvasive cervical carcinoma. Human papillomavirus (HPV) has been associated aetiologically in numerous studies with the development of cervical cancer [Kiviat, 1996; Reitano, 1997]. Furthermore, patients infected with human immunodeficiency virus (HIV) have an increased prevalence of CIN lesions compared with HIV-negative matched women [Boccalon et al., 1996]. Certain HPV types (16, 18, 31, 33, 35, 45, 52, 58) are associated specifically with cervical dysplasia and cervical cancer [Baker and Tyring, 1997]. Treatment of preinvasive or microinvasive cervical lesions is based mostly on partial resection of the cervix. The present study was a trial of treatment of CIN III lesions with local application of cidofovir (CDV), an acyclic nucleoside phosphonate derivative known to have a broad-spectrum activity against different families of DNA viruses, including HPV [Snoeck et al., 1995, 1997, 1998a, 1998b; Van Cutsem et al., 1995; Lalezari et al., 1997; Safrin et al., 1997].

PATIENTS AND METHODS

Fifteen women attending routine gynaecological screening who were found to be CIN III positive after histology on biopsy and confirmed to be HPV positive by the polymerase chain reaction (PCR) entered the study. Their mean age was 32.5 years (range 21–48). Ten were known to be smokers. All were HIV seronegative.

Informed consent was obtained from patients before entry into the study. Treatment consisted of three applications, every other day, of 3 g CDV 1% gel on the cervix under colposcopic view by the gynaecologist. The CDV 1% gel was provided by Gilead Sciences (Foster City, CA). Colposcopy, including the application of 5%

*Correspondence to: Dr. R. Snoeck, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium.

Accepted 11 June 1999

TABLE I. Patient Characteristics*

Patients		Before treatment			After treatment, at conisation			Ki 67 index	
No.	Age (years)	Histology	PCR-HPV	Colposcopy	Histology	PCR-HPV	Colposcopy	Pre-treatment (%)	Post-treatment (%)
1	36	CIN III	+	D	CINIII(neck)	+	D	44	33
2	37	CIN III	+	P	–	–	N	28	13
3	35	CIN III	+	NA	CINIII(neck)	+	NA	19	37
4	30	CIN III	+	P	CINIII	+	D	25	40
5	35	CIN III	+	P	CINIII(neck)	+	P	46	39
6	30	CIN III	+	P	–	–	NA	24	21
7	25	CIN III	+	P	–	+	D	11	17
8	37	CIN III	+	D	CINIII	+	D	40	60
9	21	CIN III	+	N	–	–	N	/	/
10	38	CIN III	+	P	CIN I	+	D	33	17
11	35	CIN III	+	P	CINIII	+	P	/	/
12	27	CIN III	+	P	–	+	P	31	17
13	22	CIN III	+	P	CINIII	+	P	25	27
14	48	CIN III	+	D	–	+	N	19	16
15	32	CIN III	+	P	–	–	N	50	20

*CIN, cervix intraepithelial neoplasia; PCR, polymerase chain reaction; HPV, human papillomavirus; P, presence of acetowhite lesion, typically suggestive of CIN; D, doubtful presence of atypical (transformation/metaplasia) zones; N, no visible lesion; ND, not determined.

acetic acid and Lugol (Schiller's test) was carried out at the first visit, before each application of CDV and immediately before conisation. Conisation was carried out no later than 4 weeks after the last CDV application. The presence of HPV on the cone and on the initial biopsy was determined by using PCR (MY09 and MY011) with L1 consensus primers as described by Ting and Manos [1990].

Ki 67 and immunohistochemistry evaluations were carried out on the biopsy samples by using the MIB-1 antibody (Immunotech, dilution 1/100) as described previously. The MIB-1 antibody is a monoclonal antibody that detects a formalin-resistant epitope of the Ki 67, a nuclear matrix cell protein that is expressed by all cycling cells, but not in non-cycling cells [Gerdes et al., 1984, 1992]. For the apoptosis index, May–Grunwald Giemsa staining and the TdT-mediated dUTP nick end labelling (TUNEL) technique [Sangiuolo et al., 1994] were used.

Routine blood analyses for haematology and biochemistry were carried out before treatment and conisation.

RESULTS

The characteristics of the patients and their response to CDV treatment are shown in Table I. The pathology on the initial biopsy for each patient did not show healthy margins, indicating that the tumour was not removed completely.

All patients received three applications (every other day) of 3 g of CDV 1% gel. The routine haematological and biochemistry tests carried out before and after CDV treatment were normal for all, except for one patient who had slightly elevated bilirubin levels before and during treatment. Conisation was done at a mean of 17 days (range 10–25) after start of the treatment. There were no particular problems during surgery, except for one patient who bled peri-operatively due to local failure of haemostasis.

In 7 of the 15 patients, a complete response was noted, characterized by the absence of residual lesions or by the presence of repair atypia in regenerative epithelium. In 4 of the 7 patients, the PCR carried out on removed cervix was negative, whereas 3 remained positive. Two patients (nos. 4 and 13) did not respond: CIN II-III lesions persisted at the surface as well as in the depth of the glands, and HPV-PCR remained positive. In 1 patient, CIN III lesions evolved to a low grade (CIN I) lesion.

In the five remaining patients a limited response was noted. The CIN II-III lesions at the superficial epithelial layers were lost, but there was persistence deep in the glands. In one of these patients, there was a focal lesion in the endocervix, whereas two had persistent CIN III at the level of the glands neck. These five patients also had evidence of HPV by PCR.

The apoptotic index, at the level of the lesions (data not shown) measured before therapy and after surgery did not show significant differences in any of the patients. The apoptotic index ranged from < 1% to 3%. The proliferation index (Ki 67) before treatment was not predictive of the clinical response. In addition, there was no correlation between the proliferation index post-surgery and the clinical response.

The activity of cidofovir was restricted to the areas that were stained initially by acetic acid (Fig. 1). As shown by Lugol staining (Fig. 2), no toxicity was observed in the normal tissue, particularly no microulcerations or signs of irritation were detected colposcopically. None of the patients mentioned any pain or discomfort during or after application of the CDV 1% gel.

DISCUSSION

Cervical cancer is the most prevalent tumour in developing countries and the second most frequent cancer in women worldwide. Specifically, HPV types 16, 18, and other high-risk HPV types have been associated aetiologically with this malignancy. HPV DNA is de-

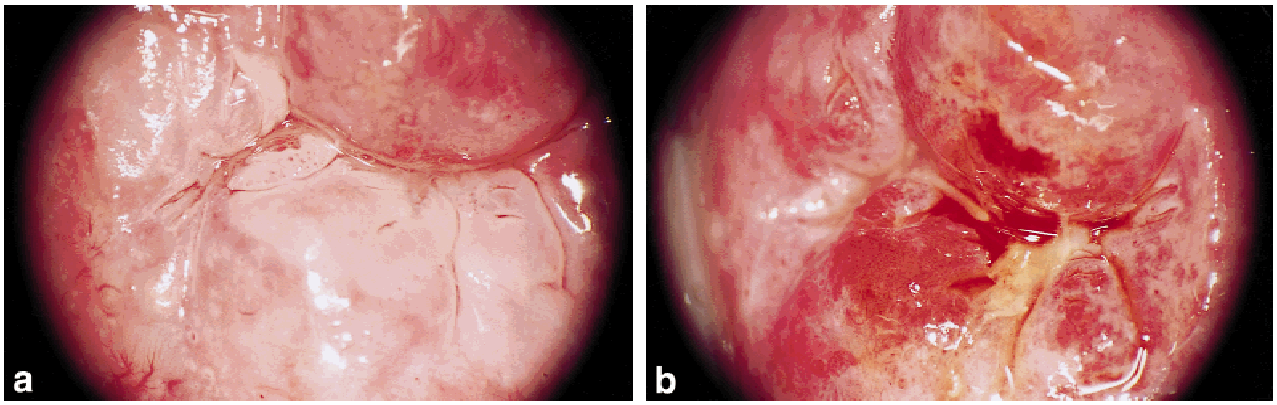

Fig. 1. Cervix stained with acetic acid **(A)** before application of cidofovir (CDV) 1% gel and **(B)** after three applications of CDV 1% gel.

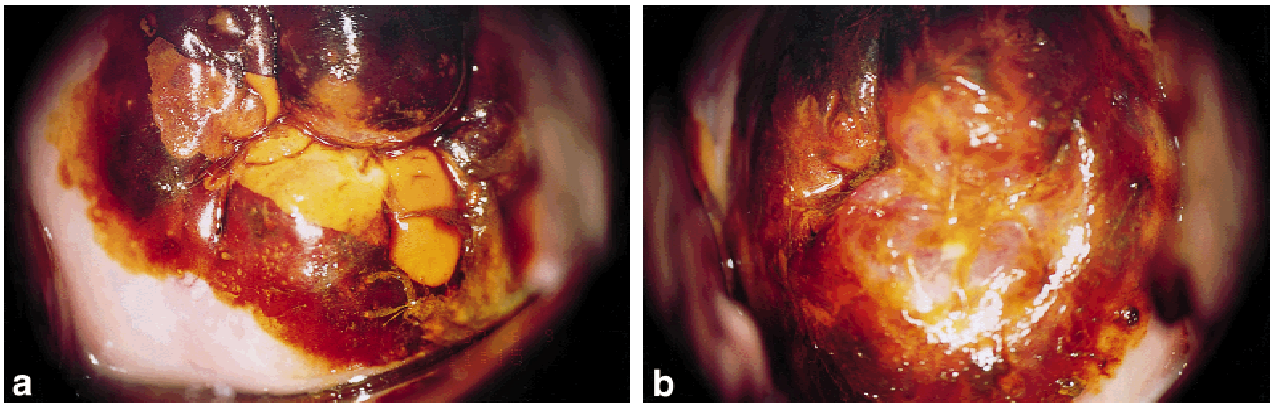

Fig. 2. Cervix stained with Lugol **(A)** before application of cidofovir (CDV) 1% gel and **(B)** after three applications of CDV 1% gel.

tected in 90% or more of the cases of CIN and invasive carcinoma [Ambros and Kurman, 1990]. Although HPV alone is probably sufficient for the development of CIN, other co-factors such as cigarette smoking, other sexually transmitted diseases (STD) (Chlamydia, Trichomonas, herpes simplex), and immunodeficiency status may be involved.

The treatment of CIN is based currently on surgery, which allows precise and complete tissue eradication [Baggish et al., 1989]. Cure rates exceeding 95% are achieved routinely regardless of disease location or histology [Wright and Chapman, 1992]. Recently, it was shown that the rate of invasive cervical cancer after treatment of CIN depends on the effectiveness of the primary treatment and the rigor of follow-up. Annual cytological follow-up is suggested for at least 10 years after treatment. The risk of developing invasive cancer after conservative treatment of CIN is low, but nevertheless four to five times greater than that in the general population, and, furthermore, with no risk reduction over time [Soutter et al., 1997]. Therefore, a follow-up of the patients treated surgically is mandatory and the use of HPV-PCR is more sensitive than cytology alone for identifying response [Chua and Hjerpe, 1997]. The complete destruction of malignant tissue by laser must be replaced by surgical excision (laser, loop electrosurgical excision procedure, cold knife), allowing adequate histo-pathological examination of all excised tissue including the entire transformation zone and 5 mm of the endocervical canal [Békássy, 1997].

Nevertheless, this treatment, particularly in young women, leads to higher incidence of obstetric problems during subsequent pregnancy [Hagen and Skjeldestad, 1993]. Other approaches have been considered such as interferon-β given directly intratumourally and/or systemically for patients diagnosed with CIN III. The highest frequency of complete response was observed in patients receiving combined intramuscular and intralesional treatment. Sixty percent of the patients treated with the combined therapy had a complete response, compared with 50% and 42% for those treated intralesionally or intramuscularly, respectively [Rotola et al., 1995].

Interferon-β given intramuscularly induced regression of CIN II in 36% of the patients, as compared with none in the placebo group [De Aloysio et al., 1994]. Interferon-β injected intralesionally in patients with CIN has led to an 80% cure rate [Penna et al., 1994]. In both studies no major side effects were recorded. Interferon-α 2b, intralesionally in a small trial, induced 33% complete remissions 1 year after treatment [Stellato, 1992]. In advanced cervical cancer, interferon-α had only minimal activity [Wadler et al., 1995]. Intramuscular interferon-α 2b injected in combination with 13-

cis-retinoic acid showed preliminary encouraging results in women with CIN II or CIN III who refused surgery [Toma et al., 1996].

Brodman et al. [1992] combined laser and topical 5-fluorouracil (5-FU) for the treatment of lesions of the vagina and the cervix, CO_2 vaporisation being followed by eight weekly applications of 5-FU. About 60% of the cervical lesions disappeared and, interestingly, all the failures had high-grade virus subtypes (16, 18, 32, 35, 51). Using a cervical cap and 5% 5-FU cream, a limited study [Davila and Shroyer, 1996] demonstrated that six of the seven patients treated had a negative PCR by 6 weeks after application and no side effects (vulvar pain or dysuria) were observed. However, application of 5-FU combined or not with carbon dioxide laser treatment, can lead to vaginal adenosis [Bornstein et al., 1993; Dungar and Wilkinson, 1995].

Recently, an open-label study demonstrated that patients with moderate cervical dysplasia respond to oral administration of β-carotene [Manetta et al., 1996]. Nevertheless, a double-blind randomised trial [Romney et al., 1997] failed to demonstrate any benefit of β-carotene supplementation in the treatment of CIN. Also, a recent phase I trial established the conditions for further studies of photodynamic therapy using dihematoporphyrin applied topically for the treatment of CIN [Monk et al., 1997].

In the present study, CDV, a nucleotide analogue with broad-spectrum activity against DNA viruses, including HPV, was shown to suppress specifically lesions characteristic of CIN III. CDV had already been shown to have selective anti-papillomavirus activity in both animal models [Kurtzman et al., 1993] and clinical trials [Snoeck et al., 1995, 1997, 1998a, 1998b; Van Cutsem et al., 1995]. The results of the present study confirm that CDV has selective activity against HPV-induced epithelial proliferation. The colposcopic pictures with acetic acid or Lugol, as well as the histology after treatment confirmed the selective suppressive effect of CDV on the proliferation of tumour cells, as compared with that of normal epithelium. Except for two (patients 4 and 13), patients had at least a partial response, and half had a total response. For those that failed partially, the tumour cells persisted only deeply in the glands. Due to imperfect bioavailability, the product may not reach the deeper layers of the tumour. Therefore, either the formulation or the schedule and/or mode of administration of the compound may be modified to as to permit better accessibility of the deeper epithelial layers to the compound.

In contrast to other non-surgical approaches for CIN III treatment, such as 5-FU or interferon, CDV acts specifically on the HPV-containing proliferating cells. The applications of CDV appeared to be well tolerated and no subjective complaints were reported. CDV should be evaluated further for the treatment of CIN III, and possibly also CIN I and CIN II dysplasias, alone or in combination with other treatment or prophylactic options such as specific HPV vaccination.

ACKNOWLEDGMENTS

We thank C. Callebaut, I. Aerts, and D. Brabants for their dedicated editorial help.

REFERENCES

Ambros RA, Kurman RJ. 1990. Current concepts in the relationship of human papillomavirus infection to the pathogenesis and classification of precancerous squamous lesions of the uterine cervix. Semin Diagn Pathol 7:158–172.

Baggish MS, Dorsey JH, Adelson M. 1989. A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser. Am J Obstet Gynecol 161:60–68.

Baker GE, Tyring SK. 1997. Therapeutic approaches to papillomavirus infections. Dermatol Clin 15:331–340.

Békássy Z. 1997. Long-term follow-up of cervical intraepithelial neoplasia treated with minimal conization by carbon dioxide laser. Lasers Surg Med 20:461–466.

Boccalon M, Tirelli U, Sopracordevole F, Vaccher E. 1996. Intraepithelial and invasive cervical neoplasia during HIV infection. Eur J Cancer 32A:2212–2217.

Bornstein J, Sova Y, Atad J, Lurie M, Abramovici H. 1993. Development of vaginal adenosis following combined 5-fluorouracil and carbon dioxide laser treatments for diffuse vaginal condylomatosis. Obstet Gynecol 81:896–898.

Brodman M, Dottino P, Friedman F Jr, Heller D, Bleiweiss I, Sperling R. 1992. Human papillomavirus-associated lesions of the vagina and cervix. Treatment with a laser and topical 5-fluorouracil. J Reprod Med 37:453–456.

Chua K-LC, Hjerpe A. 1997. Human papillomavirus analysis as a prognostic marker following conization of the cervix uteri. Gynecol Oncol 66:108–113.

Davila GW, Shroyer KR. 1996. Topical 5-fluorouracil in the treatment of cervical human papillomavirus infection. Gynecol Obstet Invest 41:275–277.

De Aloysio D, Miliffi L, Iannicelli T, Penacchioni P, Bottiglioni F. 1994. Intramuscular interferon-beta treatment of cervical intraepithelial neoplasia II associated with human papillomavirus infection. Acta Obstet Gynecol Scand 73:420–424.

Dungar CF, Wilkinson EJ. 1995. Vaginal columnar cell metaplasia. An acquired adenosis associated with topical 5-fluorouracil therapy. J Reprod Med 40:361–366.

Gerdes J, Becker MHG, Key G, Cattoretti G. 1992. Immunohistological detection of tumour growth fraction (Ki-67 antigen) in formalin-fixed and routinely processed tissues. J Pathol 168:85–86.

Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. 1984. Cell cycle analysis of a cell proliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133: 1710–1715.

Hagen B, Skjeldestad FE. 1993. The outcome of pregnancy after CO_2 laser conisation of the cervix. Br J Obstet Gynaecol 100:717–720.

Kiviat N. 1996. Natural history of cervical neoplasia: overview and update. Am J Obstet Gynecol 175:1099–1104.

Kurtzman G, Pickel M, Christensen N, Kreider J. 1993. Phosphonate nucleoside analogs are potent anti-papillomavirus agents in animal models. Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Louisiana, USA, 17–20 October 1993, no. 1593.

Lalezari J, Schacker T, Feinberg J, Gathe J, Lee S, Cheung B, Kramer F, Kessler H, Corey L, Drew WL, Boggs J, McGuire, Jaffe HS, Safrin S. 1997. A randomized, double-blind, placebo-controlled trial of cidofovir gel for the treatment of acyclovir-unresponsive mucocutaneous herpes simplex virus infection in patients with AIDS. J Infect Dis 176:892–898.

Manetta A, Schubbert T, Chapman J, Schell MJ, Peng YM, Liao SY, Meyskens FJ Jr. 1996. β-Carotene treatment of cervical intraepithelial neoplasia: a phase II study. Cancer Epidemiol Biomarkers Prev 5:929–932.

Monk BJ, Brewer C, Van Nostrand K, Berns MW, McCullough JL, Tadir Y, Manetta A. 1997. Photodynamic therapy using topically applied dihematoporphyrin ether in the treatment of cervical intraepithelial neoplasia. Gynecol Oncol 64:70–75.

Penna C, Fallani MG, Gordigiani R, Sonni L, Taddei GL, Marchionni M. 1994. Intralesional beta-interferon treatment of cervical intra-

epithelial neoplasia associated with human papillomavirus infection. Tumori 80:146–150.

Reitano M. 1997. Counseling patients with genital warts. Am J Med 102:38–43.

Romney SL, Ho GYF, Palan PR, Basu J, Kadish AS, Klein S, Mikhail M, Hagan RJ, Chang CJ, Burk RD. 1997. Effects of β-carotene and other factors on outcome of cervical dysplasia and human papillomavirus infection. Gynecol Oncol 65:483–492.

Rotola A, Costa S, Di Luca D, Stefanon B, Villani C, Micheletti L, Montemagno U, Bolis PF, Cassai E. 1995. β-Interferon treatment of cervical intraepithelial neoplasia: a multicenter clinical trial. Intervirology 38:325–331.

Safrin S, Cherrington J, Jaffe HS. 1997. Clinical uses of cidofovir. Rev Med Virol 7:145–156.

Sangiuolo E, De Santis L, Cavicchini A, Angeloni U, Romanini C, Novelli G, Dallapiccola B. 1994. A new method for direct analysis of polymerase chain reaction-amplified human papillomavirus using DNA enzyme immunoassay. Int J Clin Lab Res 24:223–226.

Snoeck R, Andrei G, Gérard M, Silverman A, Hedderman A, Balzarini J, Sadzot-Delvaux C, Tricot G, Clumeck N, De Clercq E. 1997. Successful treatment of progressive mucocutaneous infection due to acyclovir- and foscarnet-resistant herpes simplex virus with (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC). Clin Infect Dis 18:570–578.

Snoeck R, Bossens M, Parent D, Delaere B, De Greef H, De Clercq E, Jaffe HS, McGuire B, Safrin S. 1998a. A double-blind placebo-controlled study of cidofovir gel for human papillomavirus (HPV)-associated genital warts. Abstracts of the Eleventh International Conference on Antiviral Research, San Diego, California, USA, 5–10 April 1998. Antiviral Res 37:A47.

Snoeck R, Van Ranst M, Andrei G, De Clercq E, De Wit S, Poncin M, Clumeck N. 1995. Treatment of anogenital papillomavirus infections with an acyclic nucleoside phosphonate analogue. N Engl J Med 333:943–944.

Snoeck R, Wellens W, Desloovere C, Van Ranst M, Naesens L, De Clercq E, Feenstra L. 1998b. Treatment of severe laryngeal papillomatosis with intralesional injections of cidofovir [(*S*)-1-(3-hydroxy-2-phosphonylmethoxy-propyl)cytosine]. J Med Virol 54: 219–225.

Soutter WP, de Barros Lopes A, Fletcher A, Monaghan JM, Duncan ID, Paraskevaidis E, Kitchener HC. 1997. Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia. Lancet 349:978–980.

Stellato G. 1992. Intralesional recombinant alpha 2B interferon in the treatment of human papillomavirus-associated cervical epithelial neoplasia. Sex Transm Dis 19:124–126.

Ting Y, Manos MM. 1990. Detection and typing of genital human papillomaviruses. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 356–367.

Toma S, Ragni N, Raffo P, Boselli F, Gustavino C, Rugiati S, Formelli F, Palumbo R, Rosso R, De Cecco L. 1996. Efficacy of the association of 13-cis-retinoic acid (13cRA) and alpha-interferon 2a (alpha-IFN 2a) in moderate-severe cervical intraepithelial neoplasia (CIN II-III): a pilot study. Antiviral Res 16:931–936.

Van Cutsem E, Snoeck R, Van Ranst M, Fiten P, Opdenakker G, Geboes K, Janssens J, Rutgeerts P, Vantrappen G, De Clercq E. 1995. Successful treatment of a squamous papilloma of the hypopharynx-esophagus by local injections of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine. J Med Virol 45:230–235.

Wadler S, Burk RD, Neuberg D, Rameau R, Runowicz CD, Goldberg G, McGill F, Tachezy R, Comis R, Edmonson J, Wiernik PH. 1995. Lack of efficacy of interferon-α therapy in recurrent, advanced cervical cancer. J Interferon Cytokine Res 15:1011–1016.

Wright VC, Chapman W. 1992. Intraepithelial neoplasia of the lower female genital tract: etiology, investigation, and management. Semin Surg Oncol 8:180–190.